Geographical Patterns of Allozyme Variation in *Angelica japonica* (Umbelliferae) and *Farfugium japonicum* (Compositae) on the Ryukyu Islands, Japan

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Geographical patterns of the genetic differentiation of *Angelica japonica* and *Farfugium japonicum* were analyzed using allozyme analysis. Many rare alleles were found only in the southern Ryukyus populations of *A. japonica* and *F. japonicum*. Few rare or area-unique alleles were found in the northern Ryukyus or northward populations of these plant species. Genetic diversity (expected heterozygosity) of these plant species showed loss of genetic diversity in populations of a lower altitude island. The populations in low altitude islands might have been recolonized when the islands resurfaced after submersion. A geographical cline of the genetic diversity, which decreased from the central Ryukyus populations to the northward, was found in this study. These geographical patterns, which were common for these plant species, indicate that the southern Ryukyus populations were isolated from those of other areas for a long period, and the northern Ryukyus and populations northward might have recolonized rapidly from the central Ryukyus after the last glaciation.

Key words: allozyme variation, *Angelica japonica*, *Farfugium japonicum*, molecular phylogeography, the Ryukyu Islands

Phylogeography is a field of study concerned with the principles and processes governing the geographical distributions of genealogical lineages, especially those at the intraspecific level (Avise 1998). Recently, the development of molecular techniques has enabled intraspecific phylogenetic analysis. Molecular phylogeographic studies of plants and animals in North America and Europe have revealed the geographic structure of genetic differentiation at the intraspecific level (e.g., Avise *et al.* 1987; Soltis *et al.* 1997). On the Japanese mainland, some plants distributed in temperate and alpine zones have been extensively investigated using molecular phylogeographic techniques (*Fagus crenata*: Tomaru *et al.* 1997, 1998, Fujii *et al.* 2002; *Cryptomeria japonica*: Tomaru *et al.* 1994; *Abies*: Tsumura & Suyama 1998; alpine plants: Fujii *et al.* 1997, 1999). These studies suggested that the geographical patterns of molecular variation reflect the glacial history of the corresponding area.

The Ryukyu Islands lie along the eastern margin of the East China Sea between Kyushu and Taiwan. The Ryukyu Islands are divided into three areas: the northern, central, and southern Ryukyus
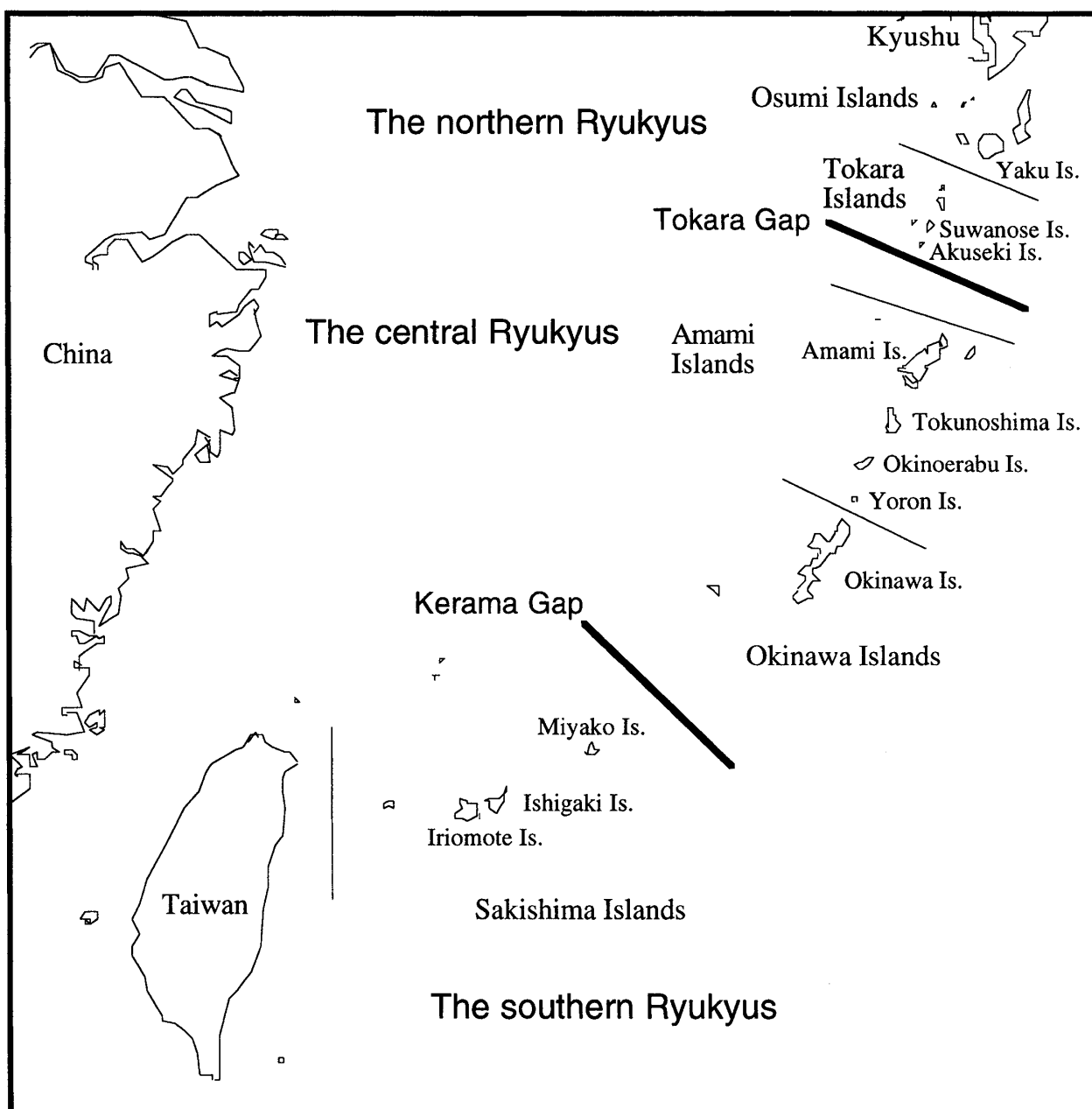
FIG. 1. Map of the Ryukyu Islands.

(Fig. 1, Mezaki 1980). About 1600 taxa have been recorded on the Ryukyu Islands (Hatusima 1980), while approximately 5000 seed plant taxa have been recorded in Japan as a whole (Satake *et al.* 1981a, 1981b, 1982, 1989a, 1989b). One third of Japanese plant species are found growing in the Ryukyu Islands. The flora of the Ryukyu Islands, like that of Taiwan, is to some extent transitional between the Holoarctic and Paleotropic floras, and

is rather strongly permeated by tropical elements, the number of which gradually increases southward (Takhtajan 1986).

Two molecular phylogeographical studies on plants of the Ryukyu Islands have been reported by different research groups (Hiramatsu *et al.* 2001; Seo *et al.* 2001). Seo *et al.* (2001) examined the genetic variation of *Peucedanum japonicum* using allozyme analysis. They reported that higher genet-

ic diversity (expected heterozygosity, *He*) was found in the central Ryukyus populations, and that the *He* values decreased in the northern populations. Seo *et al.* (2001) pointed out that these patterns of genetic variation of *P. japonicum* reflect the extinction of northern populations during the glacial period and the rapid expansion thereafter. Hiramatsu *et al.* (2001) examined the genetic differentiation of *Lilium longiflorum*, also by means of allozyme analysis. Their neighbor-joining tree of *L. longiflorum* showed three major clusters of Ryukyu populations split between Yaku Island and Amami Island; between Tokunoshima Island and Okinawan populations; and between Iriomote Island and Taiwan (Hiramatsu *et al.* 2001). They also found the depauperization in genetic diversity in the lower islands. Both of the former studies conjectured that the geographical genetic patterns found in *P. japonicum* and *L. longiflorum* populations may be highly associated with the historical geography of the Ryukyu Islands.

However, each of these studies investigated only one species or one related species group for their plant material. To be effective, a discussion on the historical process of Ryukyu flora should compare geographical patterns of genetic differentiation among several phylogenetically independent plant species. In this work, therefore, we compared the geographical genetic patterns of three independent plant species growing extensively in the Ryukyu Islands.

It is easy to imagine that plants growing in warm-temperate and subtropical zones of Japan migrate along a landbridge or island chains like stepping-stones in the area including Kyushu, Taiwan and the Chinese continent. Geographical patterns of genetic differentiation in the Ryukyu Islands and adjacent area may give us information on the formation process of warm-temperate and subtropical flora of Japan.

In addition to our previous data on *Peucedanum japonicum* Thunb. (Umbelliferae) (Seo

et al. 2001), therefore, we newly examined geographical pattern of genetic differentiation in two phylogenetically independent species, *Angelica japonica* A. Gray (Umbelliferae) and *Farfugium japonicum* (L. fil.) Kitam. (Compositae), and compared them in order to recognize any common geographic differentiation patterns in the Ryukyu Islands.

Materials and Methods

Screening of plant species with large genetic variation

At first, 19 taxa were selected from the plants distributed from southern Kyushu to the Ryukyu Islands (Table 1). Mainly, the plant species in which several intraspecific taxa or large morphological variation had been recognized from the Ryukyu Islands were selected. For each species, 10 individual samples were collected from natural populations of the following three areas: i) southern Kyushu or the northern Ryukyus (Yaku Island); ii) the central Ryukyus (Amami, Tokunoshima, or Okinawan Islands); and iii) the southern Ryukyus (Ishigaki or Iriomote Islands). These samples were analyzed using an enzyme electrophoresis method. Based on the results of this screening (Table 1), *Farfugium japonicum* and *Angelica japonica*, which had the largest genetic variation, was selected for further analyses.

Sampling plant materials

Angelica japonica (Umbelliferae) is distributed from central Honshu westward through Shikoku, Kyushu, and the Ryukyu Islands, and finally to Taiwan and the southern part of Korea (Hiroe & Constance 1958; Kitagawa 1982). The chromosome number of *A. japonica* has been reported as diploid of $2n=22$ (Arano & Saito 1977). *Farfugium japonicum* (Compositae) is distributed from central Honshu westward to the Ryukyu Islands, the southern part of Korea, and the central part of China

TABLE 1. Plant materials for screening of highly genetically variable species.

Species	Family	Life history	
Machilus thunbergii Sieb. et Zucc.	Lauraceae	tree	-
Hydrangea luteo-venosa Koidz.	Saxifragaceae	shrub	+
Hydrangea grosseserrata Engl.	Saxifragaceae	shrub	+
Hydrangea kawagoeana Koidz.	Saxifragaceae	shrub	+
Hydrangea yaeyamensis Koidz.	Saxifragaceae	shrub	+
Rubus parvifolius L.	Rosaceae	shrub	-
Callicarpa japonica var. *luxurians* Rehder	Vervenaceae	shrub	+
Clerodendrum trichotomun var. *fargesii* Rehder	Vervenaceae	shrub	-
Achyranthes bidentata Bl.	Amaranthaceae	herbaceous perennial	+
Stephania japonica (Thunb.) Miers	Menispermaceae	liana	+
Desmodium laxum ssp. *laterale* (Schindl.) Ohashi	Leguminosae	herbaceous perennial	-
Ampelopsis brevipedunculata Trautv.	Vitaceae	liana	-
Ampelopsis brevipedunculata var. *hancei* (Planch.) Rhed.	Vitaceae	liana	-
Hydrocotyle sibthorpioides Ram.	Umbelliferae	herbaceous perennial	+
Angelica japonica A. Gray	Umbelliferae	herbaceous perennial	++
Oenanthe javanica DC.	Umbelliferae	herbaceous perennial	-
Ophiorrhiza japonica Bl.	Rubiaceae	herbaceous perennial	+
Farfugium japonicum (L. fil.) Kitam.	Compositae	herbaceous perennial	+++
Adenostemma lavenia (L.) O. Kuntze	Compositae	herbaceous perennial	-
Adenostemma lavenia var. *parviflorum* (Hook. f.) Hochr.	Compositae	herbaceous perennial	-

note: +++, highest genetical variability; ++, high genetical variability; +, low genetical variability; -, no polymorphic loci.

and Taiwan (Kitamura 1981; Liu 1989). The chromosome number of *F. japonicum* has been reported as $12x$ (or $6x$) of $2n=60$ (Arano 1962; Koyama 1968). Both plant species grow near the sea.

A total of 388 fresh leaf samples from *Angelica japonica* individuals from 19 populations, and a total of 225 *Farfugium japonicum* individuals from 12 populations were collected for allozyme analyses (Fig. 2).

Enzyme Electrophoresis

About 100 mg of fresh leaf tissue was crushed in 1 ml extraction buffer (2 ml 2-mercaptoethanol, 20 mg polyvinyl-pyrrolidone, 0.1 mM Tris-HCl, 1 mM EDTA (4Na), 10 mM KCl, 10 mM MgCl$_2$, pH = 7.5). After centrifugation of homogenates at 9100 g for 10 minutes at 10℃, 20 µl of supernatant was used for electrophoresis for each enzyme. Vertical discontinuous polyacrylamide slab gel electrophoresis (Shiraishi 1988) was conducted for

genotyping of all samples. The following 14 enzyme systems were examined: alcohol dehydrogenase (ADH; EC 1.1.1.1); diaphorase (DIA; EC 1.8.1.4); glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49); glutamate dehydrogenase (GDH; EC 1.4.1.2); glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1); isocitrate dehydrogenase (IDH; EC 1.1.1.42); leuicine aminopeptidase (LAP; EC 3.4.11.1); malate dehydrogenase (MDH; EC 1.1.1.37); malic enzyme (ME; EC 1.1.1.40); 6-phosphogluconate dehydrogenase (6PG; EC 1.1.1.44); phosphoglucose mutase (PGM; EC 5.4.2.2); phosphoglucoisomerase (PGI; EC 5.3.1.9); shikimate dehydrogenase (SKD; EC 1.1.1.25); and superoxide dismutase (SOD; EC 1.15.1.1). Staining procedures followed Shiraishi (1988) and Richardson *et al*. (1986) with minor modification.

Loci were numbered in relation to their mobility; the longest distance zone was isozyme 1; the shorter distance zone was isozyme 2, 3, and so on.
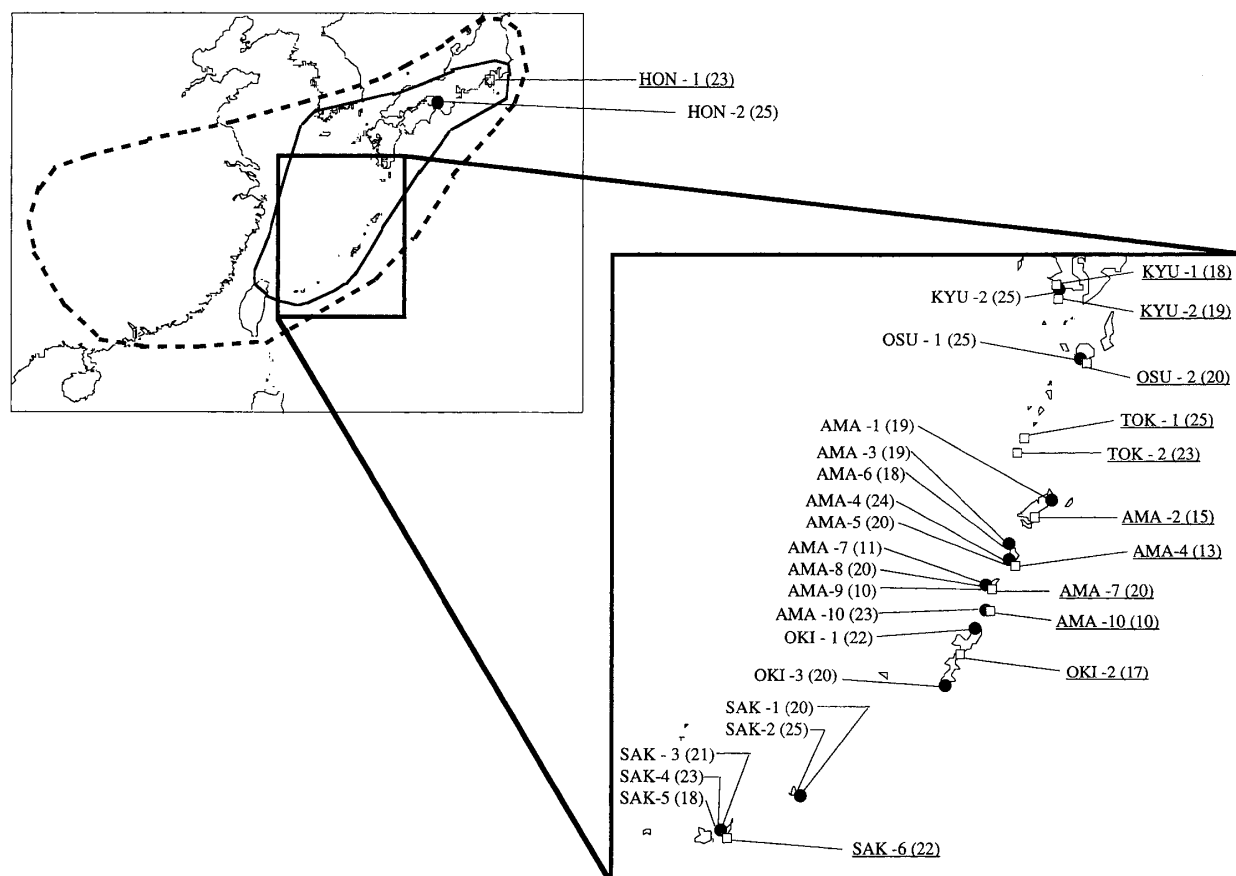
FIG. 2. Geographical distribution of *Angelica japonica* (thick line) and *Farfugium japonicum* (dotted line) and the localities of populations sampled (●, *A. japonica*; □, underlined, *F. japonicum*). Numbers in parentheses are sample size.

Allele labels alphabetically denote relative mobility, with the *a*-allele of each locus having the longest migration distance.

Data analysis

(1) Genetic variability within populations
Allele frequencies, the proportion of polymorphic loci that exhibited more than one allele (P), the mean number of alleles per locus (A), and the mean expected heterozygosities (He) were calculated for all populations of *Angelica japonica* and *Farfugium japonicum* using the program POPGENE (ver. 1.31; Yeh *et al.* 1999).

(2) Genetic differentiation among populations
Nei's (1978) genetic distance was calculated using the program POPGENE, and a UPGMA dendrogram was constructed using the program PHYLIP (ver. 3.57; Felsenstein 1995).

(3) Geographical pattern of rare alleles
UPGMA dendrograms based on allele frequencies show only genetic similarity among the populations. The hypothesis that levels of gene flow account for the distributions of rare alleles (with an overall frequency of less than 5.0% in each species) seems the most parsimonious, since it requires far fewer assumptions to explain the patterns (Slatkin 1985). We compared the geographical patterns of rare allele distribution as well as those of genetic similarity for the *Angelica japonica*, *Farfugium japonicum* and *Peucedanum japonicum* (Seo *et al.* 2001) populations.

TABLE 2. Allele frequencies of *Angelica japonica* populations. N indicates sample size.

		Awaji Is.	Makurazaki	Yaku Is.	Amami Is.		Tokunoshima Is.		
		HON-2	KYU-2	OSU-1	AMA-1	AMA-3	AMA-4	AMA-5	AMA-6
Dia-1	a								
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Got-1	a								
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	c								
Got-2	a								
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lap	a								
	b								
	c						0.23	0.35	
	d	1.00	1.00	1.00	1.00	1.00	0.77	0.63	1.00
	e						0.02		
Pgi	a	0.10	0.18	0.10	0.00	0.50	0.15	0.18	0.06
	b								
	c	0.90	0.82	0.90	1.00	0.50	0.85	0.83	0.94
	N	25	25	25	19	19	24	20	18

Results

Angelica japonica

The seven enzyme systems resolved were interpreted as being encoded by 12 loci. Of the 12 loci examined, no variations were detected in *Adh*, *G6pd-2*, *Gdh-2*, *Dia-2*, or *Dia-3*. Seven of 12 loci were polymorphic. Because *G6pd-1* and *Gdh-1* were not well resolved, they were not scored. Allele frequencies of *Angelica. japonica* are given in Table 2. *Lap* has five alleles, which was the highest number of alleles among the investigated loci. Three alleles were detected at *Got-2* and *Pgi*, and two alleles at *Got-1* and *Dia-1*. The allele frequencies at *Got-1* and *Got-2* were distinct between the northern and southern populations.

Farfugium japonicum

The seven enzyme systems resolved were interpreted as being encoded by 13 loci. Of the 13 loci examined, no variations were deteted in *Adh* and *Sod*. Eleven of 13 loci were polymorphic. Because *Dia-1*, *Dia-2*, *Gdh*, *Got-3* and *Idh* were not well resolved, they were not scored. Allele frequencies of *Farfugium japonicum* are given in Table 3. Fixed three-banded pattern of PGI was observed in the northern populations of *F. japonicum*, and sixed-banded pattern was observed in the southern polymorphic populations (Fig. 3). These patterns strongly suggested presence of the two *Pgi* loci. We called these loci *Pgi-1* and *Pgi-2*. Bands in the intermediate zone of the PGI isozymes were interpreted as interlocus heterodimers, and thus they were not scored. Six alleles were detected at *Pgi-1*, five alleles at *Got-1*, four alleles at *Lap-1* and *Lap-2*, and three alleles at *Got-2* and *Pgi-2*.

Geographic pattern of rare alleles

Two distribution patterns of rare alleles were found in *Angelica japonica*: 1) some rare alleles, i.e., *Dia-1a*, *Got-1c*, *Lap-c*, *Lap-e*, and *Pgi-b*, were found only in the Tokunoshima Island, Okinoerabu Island, or Yoron Island populations (the central Ryukyus); 2) the other rare alleles, *Lap-a* and *Lap-b*, were found only in three Ishigaki Island populations (the southern Ryukyus) (Table 2). No area-unique alle-

TABLE 2. (continued)

		Okinoerabu Is.			Yoron Is.	Okinawa Is.		Miyako Is.		Ishigaki Is.		
		AMA-7	AMA-8	AMA-9	AMA-10	OKI-1	OKI-3	SAK-1	SAK-2	SAK-3	SAK-4	SAK-5
Dia-1	*a*	0.23	0.05									
	b	0.77	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Got-1	*a*				1.00	0.14	1.00	0.98	1.00	1.00	1.00	1.00
	b	0.82	1.00	1.00		0.86		0.03				
	c	0.18										
Got-2	*a*			0.05	1.00	0.18	1.00	1.00	1.00	1.00	1.00	1.00
	b	1.00	1.00	0.95		0.82						
Lap	*a*									0.19	0.43	0.28
	b									0.48	0.22	0.22
	c		0.13									
	d	0.91	0.87	1.00	1.00	1.00	1.00	1.00	1.00	0.33	0.35	0.50
	e											
Pgi	*a*		0.03	0.20	0.04							
	b	0.45	0.37	0.15	0.09							
	c	0.55	0.61	0.65	0.87	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	N	11	20	10	23	22	20	20	25	21	23	18

les or rare alleles were found in the northern Ryukyus or northward populations.

In the *Farfugium japonicum* populations, four distributional patterns of rare alleles were found: 1) four rare alleles (*Got-1a*, *Got-1e*, *Lap-1a*, *Lap-2d*) were distributed to populations of the Tokara Islands and northward; 2) five rare alleles (*Pgi-1b*, *Pgi-1c*, *Pgi-1e*, *Pgi-1f*, *Pgi-2a*) were found on the Amami Islands and Okinawan Islands (the central Ryukyus); 3) three rare alleles (*Got-1d*, *Got-2a*, *Got-2b*) were

distributed only to the Ishigaki Island population (the southern Ryukyus); 4) three rare alleles (*Lap-1b*, *Lap-2a*, *Lap-2c*) were widely distributed over many populations (Table 3).

Genetic variation within populations

The levels of genetic variation within populations of the two species are shown in Table 4.

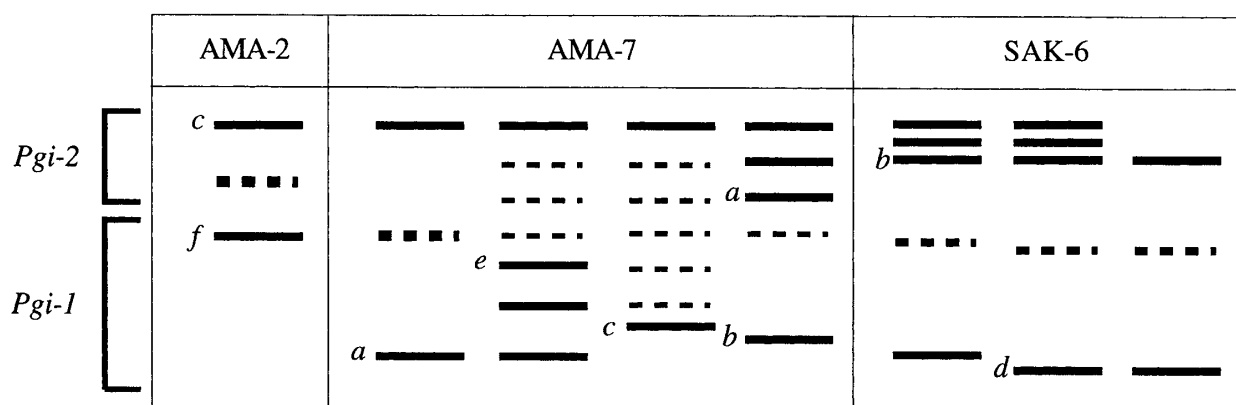*Angelica japonica*: The value of A ranged from 1.00 at OKI-3 and SAK-2 to 1.80 at AMA-7, and

FIG. 3. Zymograms showing electrophoretic patterns of phosphoglucoisomerase observed in *Farfugium japonicum* populations. Dashed lines indicate the positions of intergenic heterodimers.

TABLE 3. Allele frequencies of *Farfugium japonicum* poopulations. N indicated sample size.

		Izu HON-1	Isoma KYU-1	Makura-zaki KYU-2	Yaku Is. OSU-2	Akuseki Is. TOK-2	Suwa-nose Is. TOK-1	Amami Is. AMA-2	Tokuno-shima Is. AMA-4	Okino-erabu Is. AMA-7	Yoron Is. AMA-10	Okinawa Is. OKI-2	Ishi-gaki Is. SAK-6
Got-1	a			0.08									
	b					0.24	0.42	0.40	0.69			0.09	
	c	1.00	0.94	0.84	0.83	0.76	0.58	0.60	0.31	1.00	1.00	0.91	0.86
	d												0.14
Got-2	a												0.25
	b												0.41
	c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.34
Lap-1	a				0.08								
	b				0.10		0.12	0.10	0.23			0.06	0.11
	c		0.08	0.08		0.26	0.14	0.13	0.27	0.33	0.50	0.68	0.61
	d	1.00	0.92	0.92	0.83	0.74	0.74	0.77	0.50	0.68	0.50	0.26	0.27
Lap-2	a				0.03	0.02	0.04	0.03			0.10		
	b	0.78	0.97	1.00	0.90	0.87	0.92	0.97	0.96	1.00	0.90	1.00	0.98
	c	0.20	0.03		0.08	0.04	0.02		0.04				0.02
	d	0.02				0.07	0.02						
Pgi-1	a	1.00	1.00	1.00	1.00	1.00	1.00	0.77	1.00	0.93	1.00	0.53	0.38
	b							0.07				0.47	
	c									0.05			
	d												0.62
	e							0.10		0.03			
	f							0.07					
Pgi-2	a							0.20	0.08	0.10			
	b											0.15	0.74
	c	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.92	0.90	1.00	0.85	0.26
N		23	18	19	20	23	25	15	13	20	10	17	22

the highest value was found in the Amami Islands populations (AMA-7 and AMA-8). The same tendency was observed for P and He. P ranged from 0.00 at OKI-3 and SAK-2 to 0.44 at AMA-7. The highest value of He (0.275) was found in the Ujiji population (AMA-7).

Farfugium japonicum: The highest values of A, P and He values were found in the Ishigaki Island population (SAK-6).

Genetic divergence

UPGMA dendrograms of each species based on Nei's genetic distance are shown in Fig. 4. The dendrogram of *Angelica japonica* generated two major clusters: (1) Honshu (HON-2), Kyushu (KYU-2), Yaku Island (OSU-1), Amami Island (AMA-1), Tokunoshima Island (AMA-3, 4, 5, 6), Okinoerabu Island (AMA-7, 8, 9), and northern part of Okinawa Island (OKI-1), (2) Yoron Island (AMA-10), southern part of Okinawa Island (OKI-3), Miyako Island (SAK-1, 2), and Ishigaki Island (SAK-3, 4, 5). In cluster 2, a minor genetic differentiation between the populations of Miyako Island and Ishigaki Island was observed.

The UPGMA dendrogram of *Farfugium japonicum* generated two major clusters: (1) Honshu (HON-1), Kyushu (KYU-1, 2), Yaku Island (OSU-2), Suwanose Island (TOK-1), Akuseki Island

TABLE 4. Comparison of genetic variabilities in terms of the three measures in populations of *Angelica japonica* and *Farfugium japonicum*; *A*, mean number of alleles per locus; *P*, the proportion of polymorphic loci; *He*, mean expected heterozygosities

Angelica japonica

	A	P	He
HON-2	1.11	0.11	0.02
KYU-2	1.11	0.11	0.03
OSU-1	1.11	0.11	0.02
AMA-1	1.00	0.00	0.00
AMA-3	1.11	0.11	0.06
AMA-4	1.22	0.22	0.07
AMA-5	1.23	0.22	0.09
AMA-6	1.11	0.22	0.01
AMA-7	1.44	0.44	0.15
AMA-8	1.44	0.33	0.09
AMA-9	1.33	0.22	0.07
AMA-10	1.22	0.11	0.03
OKI-1	1.22	0.22	0.06
OKI-3	1.00	0.00	0.00
SAK-1	1.11	0.11	0.01
SAK-2	1.00	0.00	0.00
SAK-3	1.22	0.11	0.07
SAK-4	1.22	0.11	0.07
SAK-5	1.22	0.11	0.07
mean	1.18	0.15	0.05

Farfugium japonicum

	A	P	He
HON-1	1.30	0.20	0.04
KYU-1	1.40	0.40	0.04
KYU-2	1.40	0.30	0.05
OSU-2	1.70	0.40	0.09
TOK-1	1.90	0.40	0.12
TOK-2	1.80	0.40	0.12
AMA-2	2.00	0.60	0.19
AMA-4	1.70	0.50	0.15
AMA-7	1.50	0.40	0.09
AMA-10	1.50	0.30	0.08
OKI-2	1.70	0.50	0.16
SAK-6	1.90	0.70	0.27
mean	1.65	0.43	0.12

(TOK-2), Amami Island (AMA-2) and Tokunoshima Island (AMA-4), Okinoerabu Island (AMA-7), Yoron Island (AMA-10), and Okinawa Island (OKI-2), (2) Ishigaki Island (SAK-6). In cluster 1, two groups were recognized:, i) the central Ryukyus populations (AMA-2, AMA-7 and AMA-10) and northern populations, ii) the central Ryukyus populations (AMA-4 and OKI-2).

Discussion

Geographical pattern of genetic differentiation
The main purpose of this study was to recognize possible common geographical patterns of genetic differentiation among independent taxa distributed in the Ryukyu Islands. To this end, we compared the geographical patterns of allozyme variation among *Angelica japonica*, *Farfugium japonicum*, and

Peucedanum japonicum, finding common geographical patterns among them. The following two areas made common clusters on the UPGMA dendrograms (Fig. 4; Fig. 2 in Seo *et al.* 2001): I) the southern Ryukyus (Yaeyama Islands); II) the northern Ryukyus and northward, and a part of the central Ryukyus. The geographical distribution patterns of rare alleles in the three plant species also indicated high gene flows within cluster. An additional common geographical pattern was recognized between two of the three examined species, *A. japonica* and *P. japonicum*: III) the southern Ryukyus (Miyako Island), the populations of the central Ryukyus which did not show genetic affinity to the northern Ryukyus made a cluster. It seems that the seeds of these three plant species can be dispersed by wind or sea currents judging from the dispersal modes of related plant groups (Ridley 1930), and that the
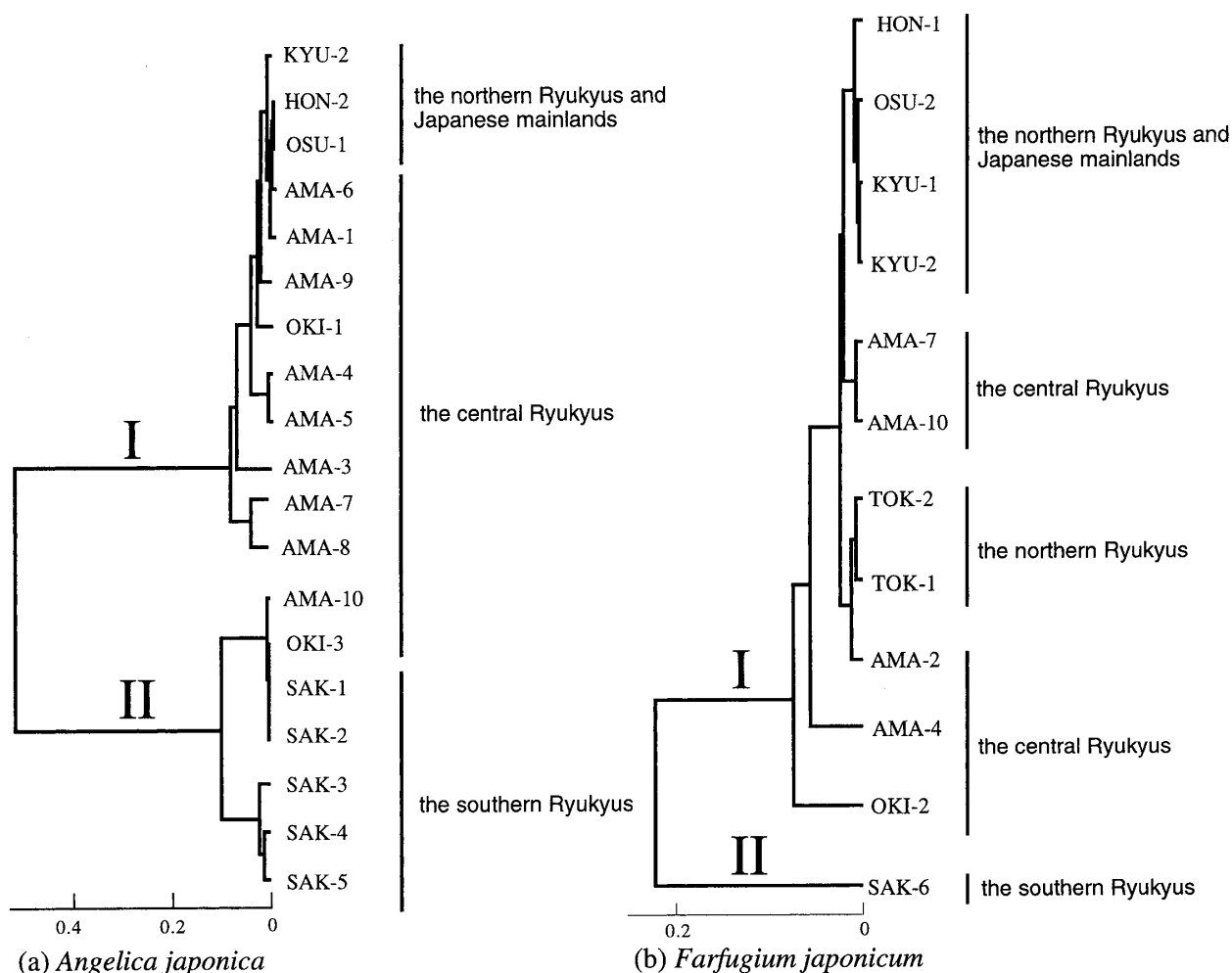
KYU-2
HON-2   the northern Ryukyus and
OSU-1   Japanese mainlands

AMA-6
AMA-1
AMA-9
OKI-1
AMA-4
AMA-5   the central Ryukyus
AMA-3
AMA-7
AMA-8

I

AMA-10
OKI-3
SAK-1
SAK-2
II
SAK-3   the southern Ryukyus
SAK-4
SAK-5

0.4   0.2   0

(a) *Angelica japonica*

HON-1
OSU-2
             the northern Ryukyus and
KYU-1   Japanese mainlands
KYU-2

AMA-7
             the central Ryukyus
AMA-10

TOK-2
             the northern Ryukyus
TOK-1

AMA-2

AMA-4   the central Ryukyus

I

OKI-2

II

SAK-6   the southern Ryukyus

0.2   0

(b) *Farfugium japonicum*

FIG. 4. Dendrograms generated by UPGMA clustering of Nei's genetic distance values.

pollen of these plants are dispersed by insects (Yahara *et al.* 1986, Seo field observation). Thus, gene flow might occur easily among the different islands. However, some instances of clear genetic differentiation were observed, even between adjacent islands- e.g., between Ishigaki Island and Miyako Island. Therefore, it is unlikely that intensive seed or pollen dispersal occurs between islands. Such low dispersibility of the these plant species might generate the above-mentioned geographic patterns of genetic differentiation. In the East China Sea, the flow of the Kuroshio Current is far away from the Ryukyu Islands (Nitani 1972). The dominant wind direction (north or south) does not coincide with the direction of island sequence in the Ryukyu Islands (northeast to southwest) (National Astronomical

Observatory 2001, Okinawa Kishodai 2000). This condition might restrict the low dispersal of these plant species between the adjacent islands. Generally, these common geographical patterns among several independent plant taxa should reflect certain types of phytogeographical events in the Ryukyu area.

In pattern I, the southern Ryukyus populations (Yaeyama Islands) of *Angelica japonica* and *Peucedanum japonicum* formed a cluster. Although only one population (SAK-6) was examined for *Farfugium japonicum*, the population was genetically well-differentiated from the northern and central Ryukyus populations. Some rare alleles were also observed only in the southern Ryukyus. It has also been reported that allozyme variation of the

Indian rice frog, *Rana limnocharis*, showed genetic differentiation between the southern Ryukyus populations and populations of the other areas in the Ryukyu Islands (Toda *et al.* 1997; Toda 1999). In the present study, therefore, we conclude that the phylogenetically independent animal and plant taxa showed similar geographical patterns of genetic differentiation. This may reflect a low gene flow between the southern Ryukyus and the other area populations, and a long period of geographic isolation from other areas.

In patterns II and III, the central Ryukyus populations have a genetic affinity both to those of the northern Ryukyus and northward, and to the southern Ryukyus. The central Ryukyus populations of *Angelica japonica* and *Farfugium japonicum* showed genetic differentiation even in the Amami Islands (Fig. 4). The populations of *Peucedanum japonicum* did not show the genetic affinity between the same areas as seen in *A. japonica* and *F. japonicum*, but still showed high genetic differentiation between the Yoron and Okinawa Islands. The Okinawa populations of *P. japonicum* were genetically similar to the Tokara Islands and northward populations (Seo *et al.* 2001). Thus, genetic differentiation was commonly observed within the central Ryukyus in the three plant species. Such a geographical pattern has been reported in many other plant and animal species (Hayashi & Matsui 1988, Hiramatsu *et al.* 2001, Maekawa *et al.* 1999, Setoguchi & Watanabe 2000, Toda *et al.* 1999, Watano & Masuyama 1994). The central Ryukyus might be a region with genetically heterogeneous populations of various geographical origin.

The central Ryukyus populations of *Angelica japonica*, *Farfugium japonicum* and *Peucedanum japonicum* had many rare alleles (Table 2, 3, Seo *et al.* 2001). The genetic-diversity parameters (A, P and He) of the three species were also high in the central Ryukyus. The islands of the central Ryukyus are relatively larger than those of the northern and southern Ryukyus (Mezaki 1980). The islands of the

central Ryukyus remained as large land masses throughout the Pleistocene (Kizaki & Oshiro 1977). Hatusima (1975) showed that the number of endemic plant taxa of the central Ryukyus was greater than that of the southern Ryukyus, and he attributed this to the fact that the islands of the central Ryukyus have a greater area, higher altitude, and older age than the other Ryukyu Islands. This heterogeneous environment and larger size of the central Ryukyus might have helped to maintain a higher genetic diversity.

Few rare alleles of *Angelica japonica*, and *Peucedanum japonicum* were distributed only in the northern Ryukyus and northward populations (Table 2, Seo *et al.* 2001). In *Farfugium japonicum*, a few rare alleles were observed also in the northern Ryukyus and northward populations, but the number of rare alleles in these areas were much less than those in the central Ryukyus populaitons (Table 3). Dendrograms for the three plant species commonly showed that populations of the northern Ryukyus and northward were genetically similar to some of the central Ryukyus populations (Fig. 4, Fig. 2 in Seo *et al.* 2001). This indicated that populations of the three plant species of the northern Ryukyus might have originated from the central Ryukyus populations.

Genetic diversity and historical biogeography of the Ryukyu Islands

A comparison of heterozygosity values (He) among *Angelica japonica*, *Farfugium japonicum*, and *Peucedanum japonicum* showed two common geographical patterns. One was loss of genetic diversity in low altitude islands and the other was a gradual decrease of genetic diversity from the central Ryukyus northward (Fig. 5). Because a large genetic differentiation between the southern Ryukyus populations of *P. japonicum* and the other populations were observed, the southern Ryukyus populations were excluded from a regression analysis of *He* and latitude of *P. japonicum* populations (Seo *et*
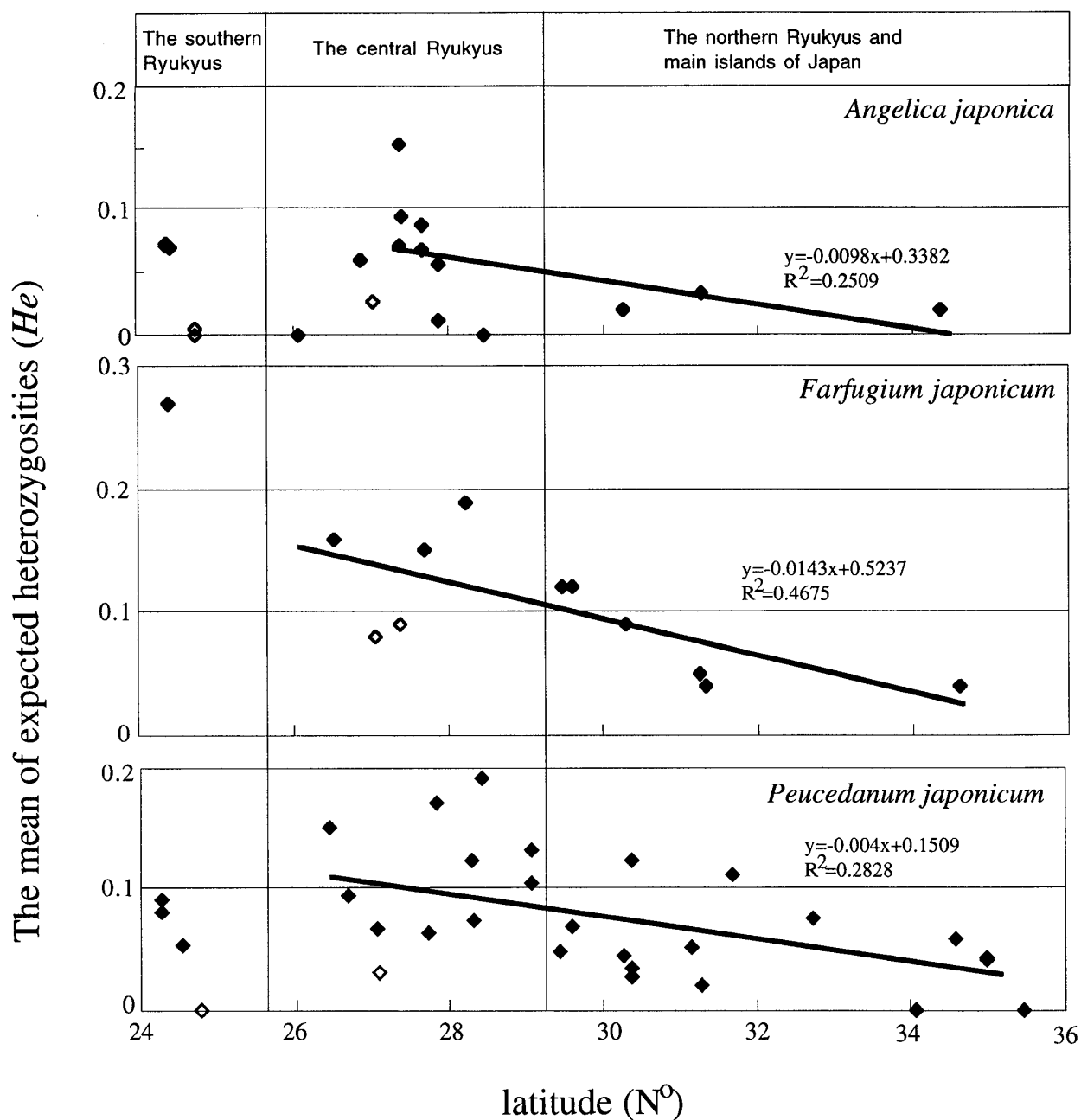
FIG. 5. Relationship between mean heterozygosities and latitude in each population of *Angelica japonica*, *Farfugium japonicum*, and *Peucedanum japonicum* (Seo *et al.* 2001). Open squares, the maximum island peak altitude of less than 200 m; black squares, more than 200 m.

al. 2001). For the same reasons, the southern Ryukyus and two of the central Ryukyus populations (AMA-10 and OKI-3) of *A. japonica* and the southern Ryukyus populations of *F. japonicum* were excluded from the regression analysis. The linear regression of *He* regarding latitude for the central Ryukyus and northward was significant (*A. japonica*, P<0.10; *F. japonicum*, P<0.05).

The Miyako Island populations of *Angelica japonica* and *Peucedanum japonicum* showed loss of genetic diversity (Table 4, Seo *et al.* 2001). A study on *Lilium longiflorum* similarly reported that the low-altitude-island populations of this plant, such as those on Yoron and Miyako islands, also tended to show lower genetic variability (Hiramatsu *et al.* 2001). This lower variability would have

resulted from the fact that lower islands were submerged during the middle Pleistocene, and their populations recolonized when they re-appeared after the late Pleistocene (Kizaki & Oshiro 1977).

He values showed a geographical cline indicating a gradual decrease of genetic diversity from the central Ryukyus populations northward (Fig. 5). Most alleles observed in the populations of the northern Ryukyus and northward were also found in those in the central Ryukyus (Table 2, 3, Seo *et al.* 2001). *He* values are strongly affected by population size (Nei 1987). Larger populations can maintain a greater amount of genetic variation and thus it is natural that larger populations have larger *He* values. If the population sizes of *Angelica japonica* and *Farfugium japonicum* in the Tokara Islands and northward were smaller than those of the Amami and the Okinawa Islands, the geographical cline of *He* observed in this study could be explained by their present population size. However, such a difference in population size of these two species was not observed in our field observation. Therefore, we considered that the *He* cline found in this study could be explained by the historical events of recent colonization. Seo *et al.* (2001) suggested that the geographical cline of genetic variability found in *Puecedanum japonicum* populations might have resulted from genetic drift or bottleneck due to the founder effect for rapid expansion to the northern populations. Considering that three phylogenetically independent plant taxa showed the same geographical cline, it is unlikely that genetic drift, which can lead to different genetic patterns, was involved in the loss of the genetic variability of these three plant species. Moreover, this is because geological events, e.g., climatic fluctuation and change of land mass, should have equally affected many taxa. The lack of genetic diversity may be related to the geological history of the Ryukyu Islands and the adjacent areas. In Europe and North America, many investigators have reported that the genetic variability in populations in non-glaciated

areas was higher than that in glaciated areas (e.g., plants: Broyles 1998, Cronberg 2000, Hewitt 1999, Lewis & Crawford 1995, Loveless & Hamrick 1988, Schwaegerle & Schaal 1979, Wolf *et al.* 1991, animals: Armbruster *et al.* 1998). The pollen fossil record showed that the distribution of warm-temperate trees was restricted to the southern end of Kyushu, Shikoku, and the Pacific sea side of Honshu (e.g., Tsukada 1983). Thus the cline of *A. japonica*, *F. japonicum* and *P. japonicum* might have been affected by rapid expansion after the glaciation period during the Pleistocine.

The geographical cline of the genetic variability and geographical distribution of allele frequencies of *Angelica japonica*, *Farfugium japonicum* and *Peucedanum japonicum* supports rapid spreading from a small number of populations in the central Ryukyus and northward after the Quaternary glacial age. The Japanese Archipelago was almost entirely covered by coniferous forests during the last glacial maximum (Tsukada 1983). The distribution of warm-temperate evergreen broadleaf forests had shifted to an area farther south than the present (Kamei & Research Group for the Biogeography from Würm Glacial 1980, Tsukada 1983). Several glacial-interglacial cycles would cause the shifting of the distribution of plants. The northern limit of the warm-temperate evergreen broadleaf forest (Tagawa 1997) and those of *A. japonica*, *F. japonicum* and *P. japonicum* are at the same latitude. During cool conditions, the northern populations of *A. japonica*, *F. japonicum* and *P. japonicum* would also have shifted to an area further south. Considering that the Tokara Gap likely became a narrow canal during the Würm Glacier (Kimura 1996), those plants might easily have moved to the central Ryukyus southward. After conditions warmed, the three species might have rapidly expanded northward. The northern populations of these plants have lower genetic diversity according to the founder effect.

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